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Original Article

Chemotaxonomic Study of Six Nigerian *Ficus* Species (Moraceae)

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Abstract

The current study was designed to investigate the chemical fingerprints in six Nigerian *Ficus* species based on the phytoconstituents for chemotaxonomic classification. The powdered leaf samples of the plants were extracted separately with absolute ethanol and chloroform and were then concentrated *in vacuole* to yield the crude ethanol and chloroform extracts respectively. The total flavonoid and tannin contents of each plant species were estimated via spectroscopic techniques. The results revealed the presence of tannins in chloroform and ethanol extracts of *F. exasperata, F. sycomorus* and chloroform extracts of *F. glumosa, F. mucuso* and *F. vogelli.* Flavonoids were present in chloroform extract of all the studied species and ethanol extract of *F. mucuso, F. exasperata* and *F. sycomorus*, while anthraquinone was absent in all. Also, saponins and alkaloids tested positive in *F. glumosa, F. vogelli, F. cordata, F. sycomorus* and *F. mucuso* (alkaloid absent), while steroids and triterpenes were present in *F. glumosa, F. mucuso, F. vogelli* and *F. exasperata* only. The presence of flavonoids, tannins, saponins, steroids and the absence of anthaquinone in all the *Ficus* species studied indicated that they are generic features. The study revealed that *Ficus* species contained valuable phytomarkers which are of pharmaceutical importance and suitable for authentication and differentiation among the studied species.

Keywords: chemotaxonomy, *Ficus* species, phytochemicals, TLC

Introduction

Plants synthesized a wide variety of bioactive compounds that are used in treatment of ailment in both humans and animals and this dates back to antiquity (Paulsamy and Jeeshna, 2011). The science of chemotaxonomy is used for the classification of plants on the basis of their chemical constituents which relied on the chemical similarity of taxon (Atal, 1982; Rasool *et al*., 2010). Three broad categories of compounds used in chemotaxonomy are primary metabolites; secondary metabolites and semantides. Primary metabolites are the compounds that are involved in the fundamental metabolic pathways (Singh, 2011).

Products of primary metabolism are usually in high volume but low value and include such substances as carbohydrates, amino acids, fatty acids and proteins which are vital for growth, development and maintenance of life processes (Rehana and Nagarajan, 2014). The products of secondary metabolism, in contrast, are usually in low volume but high value and include such substances as alkaloids, flavonoids, saponins, tannins, phenolics, steroids, terpenoids (Rehana and Nagarajan, 2014). Species in the Moraceae family have important economic and medicinal value. *Ficus* species are rich sources of bioactive secondary metabolites such as flavonoids stilbenes, triterpenoids and xanthones (Ngadjui *et al*., 2005; Han *et al*., 2006; Jayasinghe *et al*., 2008; Lee *et al*., 2009). Plants produce these chemicals to protect themselves against diseases, stress and grazing but recent research demonstrates that many of such phytochemicals can protect humans against disease. But since these secondary metabolites occur in low volume, there is therefore need to screen such medicinal plant species in order to reveal their active principles and possibly isolate and characterize them. While chromatographic techniques are unavoidably important in the separation of complex herbal mixtures, thin layer chromatography (TLC) is often the first step in identifying compounds present in such samples (Karthika *et al*., 2014). In this present study, the phytochemical constituents of six *Ficus* species (*Ficus exasperata*, *Ficus cordata*, *Ficus sycomorus*, *Ficus glumosa*, *Ficus mucuso* and *Ficus vogelii*) were investigated using Thin Layer C Chromatographic (TLC) and spectroscopic techniques.

The diversity in the genus *Ficus* has resulted in a number of taxonomic problems leading to misidentification of the

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species. These misidentifications have gross implications for the effective use of the species for their medicinal benefits. Therefore, it is envisaged that this study will provide diagnostic chemical characters for taxonomic delimitation of each of these *Ficus* species.

Materials and Methods

Collection of plant samples

Plant samples of *Ficus exasperata* Vahl., *Ficus cordata* (Warb.) C.C. Berg., *Ficus sycomorus* L., *Ficus glumosa* Del., *Ficus mucuso* Welw. Ex. *Ficalho* and *Ficus vogelii*, (Miq). Miq. were collected from various locations at Obafemi Awolowo University (Lat 7° 30 and 7° 34N, Long 4° 30 and 4°32E) Nigeria. These plants were chosen based on their ethnomedicinal information. The plants were identified and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile - Ife, Nigeria. Voucher specimens were also deposited in this herbarium.

Preparation of the extract

Each plant material was air dried and milled into powder. The powdered materials (20 g each) were separately extracted with absolute ethanol or chloroform for 48 hrs respectively and filtered. The filtrate for each sample material was concentrated in vacuole at 35 °C to yield the ethanol and chloroform extracts of each sample.

Phytochemical screening

The standard methods of (Trease and Evans, 2002) and (Sofowora, 2006) were adopted for phytochemical screening of the plant extracts. Alkaloids (Mayer's, Wagner's and Dragendorf reagents), saponins (froth test), flavonoids (ethanolic KOH/ethylacetate), cardiac glycosides (chloroform/ H_2SO_4), tannins (ferric chloride reagent), xanthoprotein (dilute H_2SO_4 /benzene /ammonia solution), anthraquinones (ethanolic NaOH), phlabotannins (HNO_3 /ammonia solution), triterpenes (chloroform/conc. H_2SO_4) and steroids (conc. H_2SO_4).

Estimation of tannins content

Tannic acid standard method (Van-Burden and Robinson, 1981) was adopted for the estimation of total tannin contents. The reaction mixture, consisted of extract (0.2 ml), 0.2 ml ammonium ferric citrate (3.5 g/l) and 0.2 ml of 20% (v/v) ammonia, was incubated for 15 min at room temperature. The absorbance was read at 500 nm against the reagent blank. The concentration of tannins in the extract was extrapolated from the standard curve and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract).

Total flavonoids content

Aluminum chloride reaction method (Sun *et al*., 1999) was adopted for the estimation of total flavonoid concentration. The reaction mixtures consisted of extract (0.2 ml) in ethanol, distilled water (2.8 ml), 5% (w/v) $NaNO_2$ (0.3 ml), 10% (w/v) $AlCl_3$ (0.3 ml) and 4% (w/v) NaOH (4 ml). The reaction mixture was allowed to stand for 15 min and absorbance was read at 500 nm. The flavonoid content of the extracts was expressed as mg/g (QE) Quercetin equivalent.

Thin layer chromatographic analysis

The ethanol and chloroform extracts of the six *Ficus* species were manually spotted using capillary tubes on precoated TLC Silica gel plates 60 F254 (MERCK, Germany) (20 × 20 cm with 0.2 mm thickness (Wagner *et al*., 1996). The plates were activated at 105 °C for 15 min and then transferred into a chromatography tank already saturated with appropriate solvent systems. After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 1% aluminium chloride, 1% ferric chloride, 50% vanillin sulfuric acid and 70% sulphuric-acetic acid in ethanol were used to identified alkaloids, flavonoid, tannins, saponin and steroid respectively. The retardation factor (Rf) was calculated from the equation:

Retardation factor (Rf) =

Distance moved by solute/Distance moved by solvent

Results

The results of phytoconstituents of chloroform and ethanolic leaf extracts of six *Ficus* species are as shown in Table 1. All the studied *Ficus* plants tested positive for flavonoids, tannins, saponins and steroids. However, anthraquinone was absent in all the plant samples; alkaloids were specific to *F. glumosa*, *F. vogelli*, *F. cordata* and *F. sycomorus* while triterpenes were present in *F. glumosa*, *F. mucuso*, *F. vogelli* and *F. exasperata*. Cardiac glycosides tested positive in *F. mucuso*, *F. vogelli*, *F. exasperata* and *F. sycomorus* while *F. cordata*, *F. vogelli*, and *F. exasperata* tested positive for xanthoproteins. Phlobatannins was present only in *F. vogelli* and *F. sycomorus*.

TLC fingerprints (Fig. 1) confirmed the presence of various phytochemicals in the studied *Ficus* species, as indicated in Table 1, after spraying with appropriate reagents (Table 2). Different Rf values (Table 3) were produced by different phytochemicals in different solvent system. Alkaloid with Rf value 0.88 is peculiar to *F. vogelli*, *F. sycomorus*, for flavonoids, while species like *F. vogelli*, *F. glumosa*, *F. cordata* and *F. sycomorus* all show congruence in having similar spots at Rf 0.70 and 0.80. Similar pattern of relationship is observed in the types of tannins, saponins and steroids with only few exceptions.

Quantitative analysis of flavonoids and tannins contents in the leaves of the studied *Ficus* species are as shown in Table 4. It was observed that chloroform extracted flavonoid better in all the studied *Ficus* species except for *F. cordata* and *F. vogelli* while ethanol showed a better solvent of extraction for tannin with exception of *F. mucuso*. The highest total flavonoid and tannins content were found in chloroform extracts of *F. glumosa* and *F. mucuso* respectively. Figures 2a and 2b show the extent of relationship among the *Ficus* species based on the ethanolic and Choroform extracts. The *Ficus* species were clustered differently based on the phytoconstituent as well as on the basis of types of extracts.

Table 1. Phytochemicals constituents of chloroform and ethanolic leaf extracts of different *Ficus* species

	Tannins	Flavo-noids	Saponins	Steroids	Cardiac glycosides	Phloba-tannins	Anthro-quinone	Triter-penes	Alkaloids	Xantho-proteins
F. glumosa^{EE}	-	-	-	-	-	-	-	+	-	-
F. glumosa^{CE}	+	+	+	+	-	-	-	+	+	-
F. mucuso^{EE}	-	+	+	-	+	-	-	+	-	-
F. mucuso^{Cz}	+	+	+	+	+	-	-	+	-	-
F. vogelli^{EE}	-	-	-	-	+	+	-	+	+	+
F. vogelli^{CE}	+	+	+	+	-	+	-	-	-	-
F. exasperata^{EE}	+	+	+	+	-	-	-	-	-	-
F. exasperata^{CE}	+	+	+	+	+	-	-	+	-	+
F. cordata^{EE}	-	-	-	+	-	-	-	-	+	-
F. cordata^{CE}	-	+	+	+	-	-	-	-	-	+
F. sycomorus^{EE}	+	+	-	-	-	-	-	-	+	-
F. sycomorus^{CE}	+	+	+	+	+	+	-	-	-	-

Table 2. Solvent system/spraying reagent used in chromatographic procedure of *Ficus* leaf extracts

S/N	Secondary metabolites	Solvent system	Solvent ratio	Spraying reagent
1	Alkaloids	EtOAc:MeOH:H_2O	10:1.4:1	Dragendorff
2	Flavonoids	Toluene:Acetone:Formic acid	4.5:4.5:1.0	1%Ethanolic $AlCl_3$
3	Tannins	EtOAc:Formic acid:MeOH	3:3:0.8:0.2	5% $FeCl_3$
4	Saponins	Chloroform:MeOH	1.2:0.2	50%Vanillin H_2SO_4
5	Steroids	Hexane: EtOAc	7.2:2.9	70% H_2SO_4- Acetic acid in ethanol

EtOAc – Ethylacetate; MeOH – Methanol; "-": Absent; "+": Present; EE: Ethanol Extract; CE: Chloroform extract

Table 3. Rf values of TLC solvent systems for leaf extract of different *Ficus* species

	Alkaloids	Flavonoids	Tannins	Saponins	Steroids
F. glumosa^{EE}	-	-	-	-	-
F. glumosa^{CE}	1.00	0.70, 0.84	0.7, 0.80	0.43, 0.75	0.09, 0.22, 0.49
F. mucuso^{EE}	-	-	0.25	-	
F. mucuso^{CE}	-	0.72, 0.88	0.38, 0.43, 0.50, 0.68	0.43, 0.50, 0.68	0.09, 0.22, 0.53, 0.78
F. vogelli ^{EE}	0.88	-	-	-	
F. vogelli ^{CE}	-	0.64, 0.86	0.94	0.38,0.40, 0.50, 0.66	0.09, 0.22, 0.80
F. exasperata^{EE}	-	0.58, 0.70	0.25	-	0.01
F. exasperata^{CE}	-	0.40, 0.58, 0.60, 0.70, 0.78, 0.86	0.15, 0.50, 0.70, 0.80	0.35, 0.40, 0.50, 0.68	0.07, 0.20, 0.56
F. cordata ^{EE}	-	-	-		-
F. cordata ^{CE}	0.12, 0.85, 0.97	0.44, 0.60, 0.70, 0.80, 0.86	0.25, 0.27, 0.38, 0.40, 0.50, 0.60	0.38, 0.45, 0.55, 0.63, 0.70	0.22
F. sycomorus^{EE}	0.88	0.56	-	-	-
F. sycomorus^{CE}	-	0.64, 0.80	0.25, 0.73, 0.80	0.40, 0.48, 0.63, 0.75	0.09, 0.36

EE: Ethanol Extract CE: Chloroform extract

Table 4. Flavonoids and tannins content of *Ficus* leaf extracts

Ficus species	Total Flavonoids mg QE/g extract	Total Tannins mg TAE/g extract
F. cordata CE	0.036±0.004	28.740±0.003
F. cordata EE	0.082±0.017	31.140±0.002
F. exasperata CE	0.096±0.026	21.248±0.037
*F. exasperata*EE	0.021±0.003	28.421±0.036
F. sycomorus CE	0.062± 0.014	29.964±0.006
F. sycomorus EE	0.048± 0.002	28.823±0.006
F. glumosa CE	0.101±0.019	33.758±0.004
F. glumosa EE	0.063±0.002	38.860±0.018
F. mucuso CE	0.063±0.002	44.166±0.001
F. mucuso EE	0.047±0.015	42.125±0.004
F. vogelli CE	0.040±0.001	34.982±0.001
F. vogelli EE	0.059±0.003	4.489±0.005

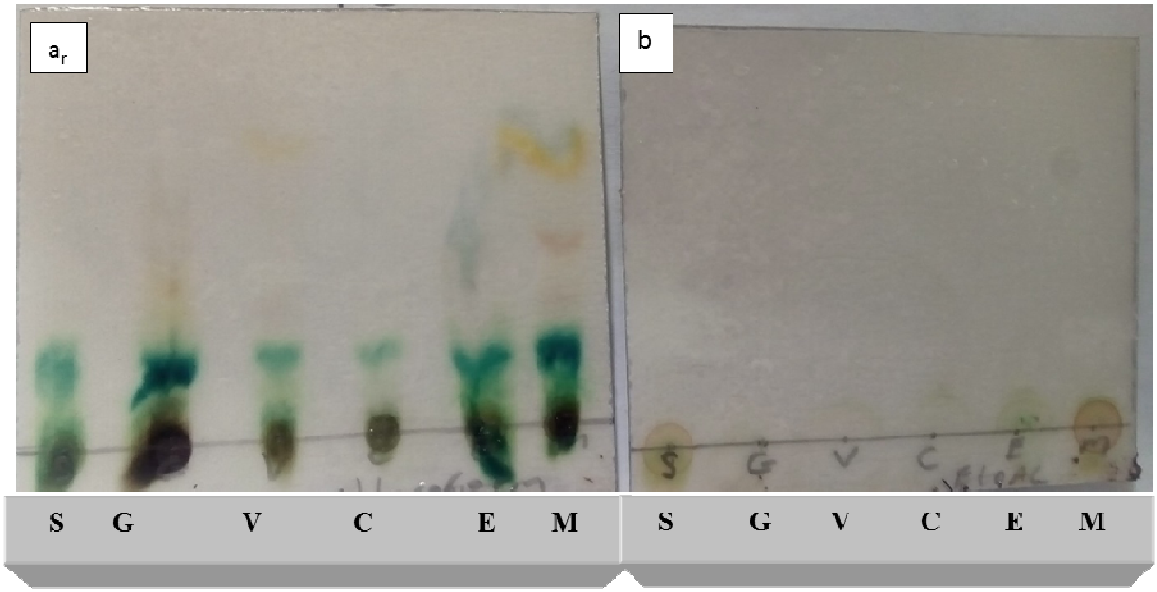

Fig. 1. TLC profile of steroids in chloroform (a) and ethanolic (b) extracts of *F. sycomorus* (S), *F. glumosa* (G), *F. vogelli* (V), *F. cordata* (C), *F. exasperata* (E) and *F. mucuso (M)*

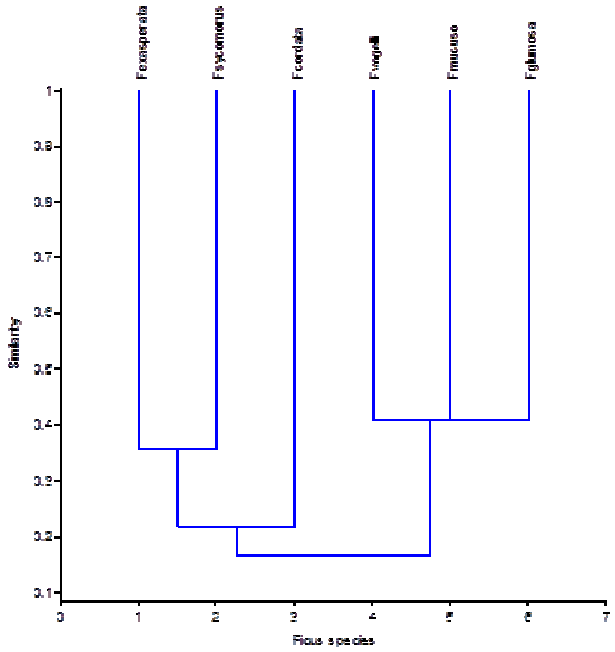

Fig. 2a. Dendrogram showing the chemotaxonomic relationship among six *Ficus* species from ethanolic extracts

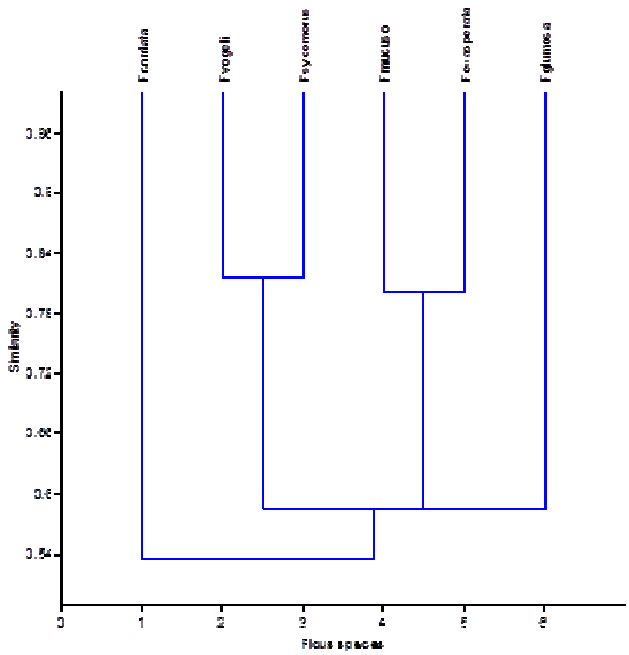

Fig. 2b. Dendrogram showing the chemotaxonomic relationship among six *Ficus* species from ethanolic extract

Discussion

A sound knowledge of the chemical constituents of plants is essential not only for the discovery of therapeutic agents but also for information on the phylogenetic relationship among the taxa under consideration. Chromatographic procedure has been reported to be one of the techniques used in methods of separation of plant metabolites (Kokate *et al*., 2006). The *Ficus* species studied showed the presence of pharmaceutically useful classes of phytochemicals.

Phytochemicals are naturally occurring and biologically active plant compounds (Halliwell and Gutteridge, 1992) that give plants their therapeutic and pharmaceutical properties (Evans, 2002). Alkaloids are group of naturally occurring products that contain mostly basic nitrogen atoms, though usually toxic to some organisms but have pharmacological agents. Most alkaloids are used as active drugs in treatment of various diseases to treat diseases such

as diabetes, cancer, cardiac dysfunction etc. and are also used as anaesthesia to relieve pain (Nicole and Cassiano, 2011).

Flavonoids constitute one of the most important groups of polyphenols used as natural antioxidant in food, medicinal and non-nutritive plant materials because of their ability to scavenge free radicals (Kim *et al*., 1990). Flavonoids had been reported to possess many useful pharmacological properties such as anti-inflammatory, oestrogenic, anti-allergic and antitumor activities (Larson, 1998; Tapas *et al*., 2008).

Saponins have been variously attributed with a diverse range of pharmacological properties such as immunostimulant, antioxidant, pesticidal, insecticidal, molluscicidal, allelopathic, protectants (Hostettmann and Marston, 1995). Some saponins are used in confectionery, as beverages and cosmetics (Price *et al*., 1987; Petit *et al*., 1995; Uematsu *et al*., 2000). Moreover, saponins and polyphenols had been documented to possessed hypocholestrolemic effects in both animals and humans which reduces incidence of atherosclerosis (John and Chapman, 1995; John, 1996).

Tannins, widely distributed among many plant species, are found in plant vacuoles and surface waxes (Devi *et al*., 2012). Studies have reported anti-inflammatory, antioxidant; antiviral, antibacterial, anti-parasitic, anticancer, antiseptic and antidiuretic properties of tannins (Souza *et al*., 2006; Banso and Adeyemo, 2007). The metabolite is also regarded to possess potential malaria suppressive effects through sequestration of iron and as botanical chelators (Etkins and Ross, 1982; Etkins, 1996). Tannin constituents had been reported to prevent lipid peroxidation by inhibiting cyclooxygenase activity (Zhang *et al*., 2004)

Steroidal compounds are of importance and play a vital role as anti-inflammatory (Cos *et al.,* 2004; Ghaderi *et al*., 2014), antibacterial, antiviral and aphrodisiac agents. Certain steroids are also used in treatment of sexual dysfunction (Sunday *et al*., 2012).

Cardiac glycosides are also pharmacological important phytochemical used in treatment of heart related diseases e.g. Digitalis and *Strophanthus* species (Seigler, 1998). Antibacterial and antifungal properties due to presence of terpenoids had also been reported (Amaral *et al*., 1998).

The TLC profiling of both the chloroform and ethanolic extracts of the studied *Ficus* species confirmed the presence of number of phytochemicals. It can be inferred from the TLC profiles that chloroform (a nonpolar solvent) extracted more varieties of phytoconstituents from the leaves of *Ficus* species than ethanol (a polar solvent).

The occurrence and variation of secondary metabolites in *Ficus* species are not only important in chemotaxonomy but also in understanding the species-function relationship. Takhajatan (1973) reported that both qualitative and quantitative information on secondary metabolites are useful in taxonomic classification of plant. The presence of flavonoids, tannins, saponins, steroids and the absence of anthaquinone in all the *Ficus* species studied indicated that they are generic features.

Moreover, there is a general overlap in the types of secondary metabolites present in all the Ficus species studied. This could possibly explain the difficulty in devising a good infrageneric classification for the genus as earlier reported by several authors. The observations recorded in the study align with the current subgeneric, sectional and sub sectional classification schemes produced by Ogunkunle and Oladele (2008) based on foliar epidermal morphology of the members of the genus.

It was evident from this study that *Ficus* species contained secondary metabolites with antioxidant activity (flavonoids and tannins). This corroborates the report by Sirisha *et al*. (2010) that *Ficus* species are rich source of naturally occurring antioxidant. Therefore, the leaves of studied *Ficus* species are potential source of natural antioxidant that could possibly boost the antioxidant defense system in humans when consumed.

The patterns of clustering observed in the dendrogram clearly contradict the sectional classification of Croner (1965) reproduced by Sonibare *et al*. (2005). The species were clustered in a way that does align with classification based on morphometric analysis as done by Sonibare *et al*., (2004).

Conclusions

The present study revealed useful information on phytoconstituent and chemotypes variation for a better identification and classification of some *Ficus* species. Researches are in progress to clarify the identity of these phytoconstituents and to provide information on authentication as well as on the taxonomic relationship existing among the *Ficus* species.

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